Reference: *Biol. Bull.*, **160**: 240–249. (April, 1981)

THE LYMPHOMYELOID (HEMOPOIETIC) SYSTEM OF THE ATLANTIC NURSE SHARK, *GINGLYMOSTOMA CIRRATUM*

RAGNAR FÄNGE AND ARTUR MATTISSON

Department of Zoophysiology, University of Göteborg, Sweden

Abstract

The lymphomyeloid system of nearly adult nurse sharks, *Ginglymostoma cirratum,* was investigated. Lymphomyeloid structures detectable by the naked eye at dissection are the epigonal organ and the spleen. Microscopic examination shows that the epigonal organ produces granulocytes and lymphocytes. The white pulp of the spleen is lymphoid, whereas the red pulp is mainly erythropoietic. Cells with the morphological characteristics of plasma cells occur in the epigonal organ and the spleen. Peroxidase-positive granulated cells are found in the epigonal organ. In contrast to many other elasmobranchs, the nurse shark lacks the Leydig organ, *i.e.* the lymphomyeloid structure of the esophagus; but the epigonal organ is well developed and averages 0.60% of the body weight. The spleen weighs about 0.26% of the body weight. The mode of life of the nurse shark in shallow tropical waters probably puts a considerable demand on its immune system. Cells produced by the epigonal organ may be important in immune responses and in inflammatory processes.

Introduction

Elasmobranch fish lack bone marrow and lymph nodes, but possess a thymus gland (at least in early life), a spleen, and voluminous lymphomyeloid tissues associated with the gonads, esophagus, or both. Diffuse lymphoid infiltrations may occur in the intestine or elsewhere. The gonad-associated lymphomyeloid tissue, often called the epigonal organ, reaches considerable size in certain sharks, such as the basking shark, *Cetorhinus maximus* (Matthews, 1950). Elasmobranch species lacking epigonal organs possess a similar tissue, termed the Leydig organ, in the esophagus (Fänge, 1977). The epigonal and Leydig organs, both relatively little known structures, resemble bone marrow and lymph nodes of higher vertebrates. Most previous studies of elasmobranch lymphomyeloid tissues concern species from temperate or cool waters. The present work deals with the lymphomyeloid system of the nurse shark, *Ginglymostoma cirratum* (family Orectolobidae), that lives in tropical and subtropical parts of the Atlantic Ocean.

Materials and Methods

Six not-fully-adult specimens of *Ginglymostoma cirratum* trapped off Puerto Rico were kept in running seawater for several weeks and fed minced molluscs. The nurse shark is one of the few sharks that can be maintained in captivity for long periods (Clark, 1963). Five sharks were killed and perfused with formalin solution. The sixth animal was investigated immediately after killing, without fixation. The main lymphomyeloid structures were isolated by dissection and weighed. Histological sections were stained with eosin-haematoxylin. Due to the paucity of

Received 14 October 1981, accepted 22 January 1981.
Abbreviation: MGG, May-Grünwald-Giemsa.

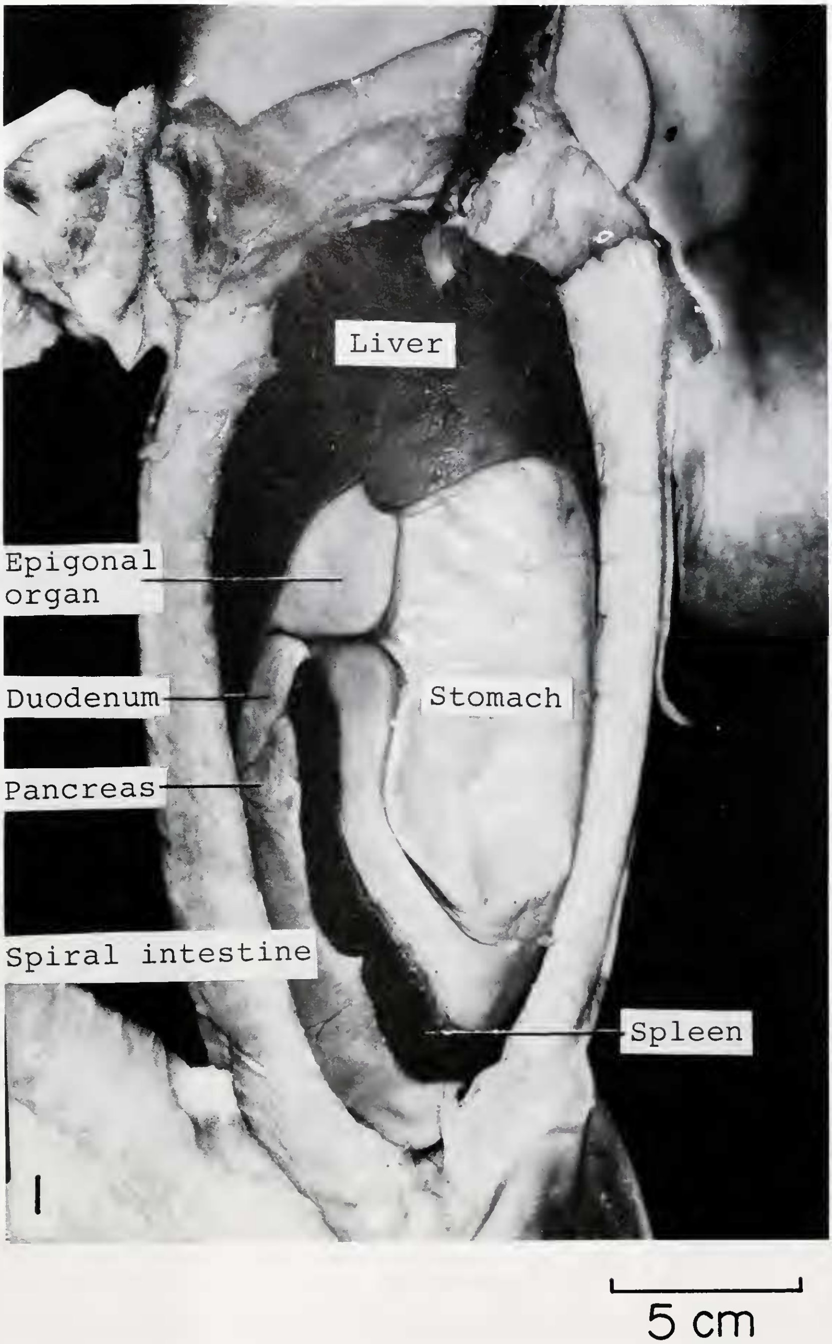
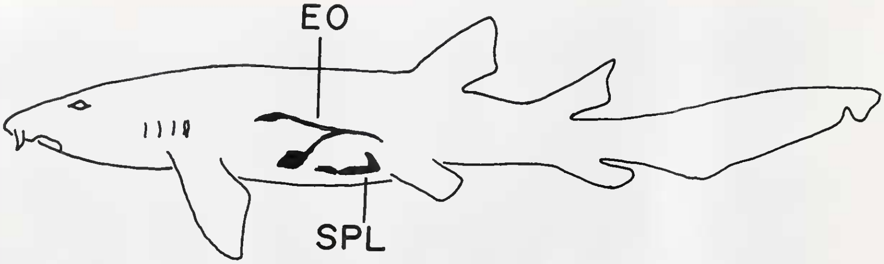
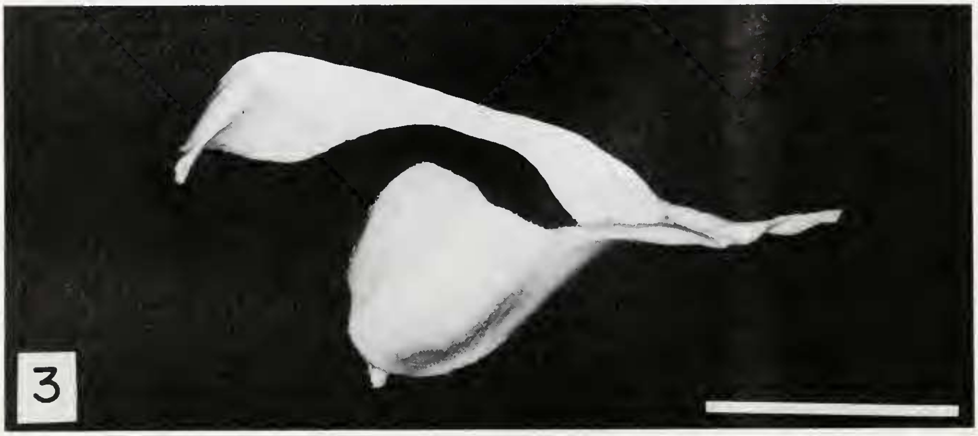


FIGURE 1. Ventral view of abdominal viscera of a female nurse shark after perfusion. The club-shaped right anterior part of the epigonal organ is in the center of the figure.



2



3

4

FIGURE 2. Diagram of the positions in the body of the epigonal organ (EO) and the spleen (SPL) of a female.

FIGURE 3. Ventral view of formalin-fixed female epigonal organ. The club-shaped right anterior lobe (low center) contains the single ovary. This is embedded in lymphomyeloid tissue and not visible. Photograph with background blacked out. Bar = 5 cm.

FIGURE 4. Ventral view of unfixed freshly dissected male epigonal organ. Testicular tissue is visible in the right anterior lobe (low left). Photograph with background blacked out. Bar = 5 cm.

fresh material, formalin-fixed material also was used for electron microscopy, after post-fixation in 1% OsO_4 dissolved in 0.1 *M* sodium cacodylate buffer, pH 7.3; and embedding in Epon 812. The sections were stained with uranyl acetate and lead citrate and examined with a Hitachi HS-8 instrument.

Table I

Weights of epigonal organ and spleen in relation to body weight (b.w.) of the nurse shark, Ginglymostoma cirratum.

No.	Sex	Body weight (g)	Epigonal organ (% b.w.)	Spleen (% b.w.)
1	Female	1930	0.79	0.21
2	Female	2311	0.43	0.22
3	Female	2750	0.62	0.29
4	Male	2897	0.79	0.36
5	Female	3800	0.42	0.26
6	Female	6700	0.57	0.24
Mean		3398	0.60	0.26

Cytochemical tests for peroxidase activity were performed on frozen sections of formalin-fixed material using the method of De Olmos and Heimer (1977).

Blood smears and imprints from the freshly investigated specimen were air-dried, fixed 15 min in methanol, and stained with May-Grünwald-Giemsa (MGG).

Results

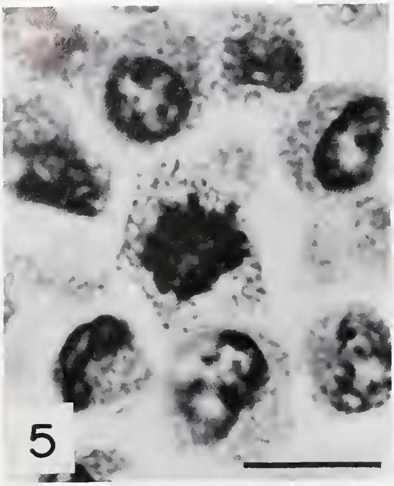
The epigonal organ and the spleen were the only lymphomyeloid structures examined that are detectable by the naked eye during dissection (Fig. 1, 2). Neither the thymus nor the Leydig organ are visible macroscopically, but a thymus undoubtedly exists during larval stages, as has been described from other species of elasmobranchs.

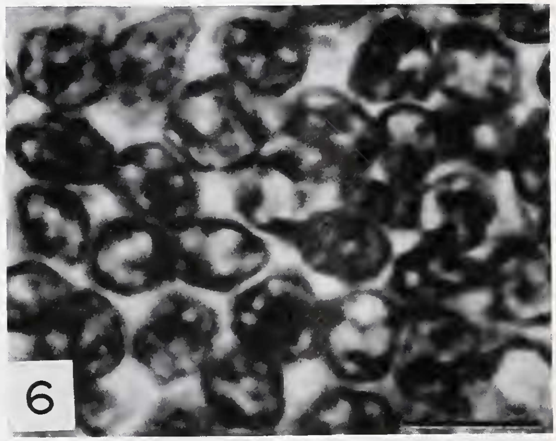
Epigonal organ

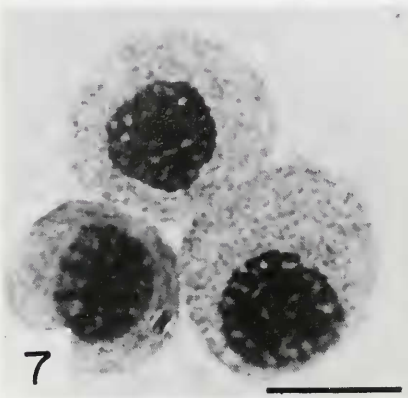
The epigonal organ, a whitish Y-shaped structure in the abdomen (Fig. 3, 4), averages 0.60% of the shark's body weight (Table I). In the male the two testes, each about the same size as the other, are embedded within the left and right anterior parts of the epigonal organ (Fig. 4). In the female the right anterior part of the epigonal organ is enlarged and club-like and contains the right ovary (Fig. 3). The left ovary is rudimentary or absent. From its dorsal attachment the right anterior part of the female epigonal organ spirals, or curves, towards the ventral side of the body cavity, forming a prominent tissue lobe between the liver and the duodenal-splenic-pyloric complex (Fig. 1). The left anterior part of the female epigonal organ is thin, elongate, and devoid of gonadal tissue. In both sexes the epigonal tissues extend caudally to the rectal gland. The sexually immature fishes examined had gonads weighing 5–10% of the corresponding epigonal organs.

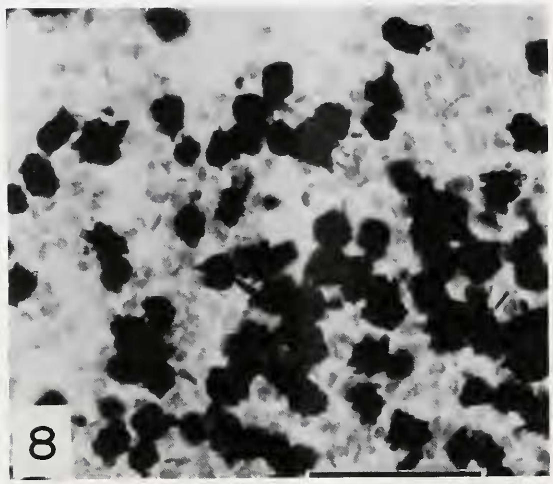
A peritoneum covers the epigonal organ. The parenchyma consists of large amounts of leucocytes, in various stages of development, in the meshes of a stroma formed by connective tissue and the walls of blood vessels and blood lacunes. Granulocytes with a round, oval, or slightly segmented nucleus (Fig. 5, 7) are the most common types of cells. The slight nuclear segmentation indicates that the granulocytes of the epigonal organ are less mature than those of the circulating blood, which usually have 2-, 3- or 4-lobed nuclei (Fig. 9). Granulocytes often undergo mitosis, probably in the promyelocyte stage (Fig. 5). Numerous cells, seemingly granulocytes, contain peroxidase-positive cytoplasmic granules (Fig. 8).

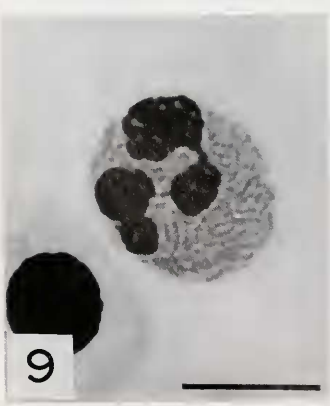
In imprint preparations the leucocytic granules appear oval, rod-shaped, or round, and are weakly eosinophilic. MGG-stained imprints show, in addition to granulocytes, numerous non-granulated cells with basophilic cytoplasm and large

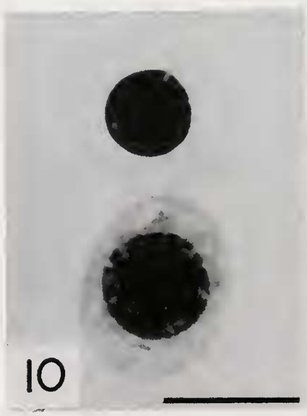












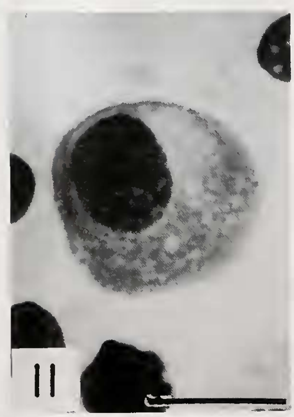


FIGURE 5. Histological section of the epigonal organ stained with eosin-haematoxylin, showing promyelocytes. One cell in mitosis. Bar = 10 μm.

FIGURE 6. Histological section of the epigonal organ stained with eosin-haematoxylin, showing follicle-like aggregation of lymphocytes. Bar = 10 μm.

nuclei. Many of these cells are granuloblasts or lymphoblasts. Other non-granulated cells, difficult to distinguish from the blast-type cells, fulfill morphological criteria for plasma cells: an eccentric nucleus with aggregations of chromatin and a strongly basophilic cytoplasm with a pale area next to the nucleus (centrosphere and Golgi apparatus). Nuclei with little or no cytoplasm around them are small lymphocytes, which are very abundant scattered among the granulocytes or in follicle-like aggregations (Fig. 6).

In the electron microscope, one or a few crystalloid rhomboid inclusions usually were seen in the granules of the granulocytes (Fig. 12).

Spleen

The spleen is of normal appearance for an elasmobranch. It is smaller than the epigonal organ, about 0.26% of the body weight (Table I). It consists of red and white pulp. White pulp (periarterial sheaths of Malpighian bodies) consists of rounded masses of densely packed lymphocytes. The diameters of the lymphoid masses vary between about 200 μm and 1200 μm. The lymphoid masses contain no granulocytes or erythrocytes; but blast-type cells, probably lymphoblasts, are scattered among large numbers of small lymphocytes. A few small arteries and capillaries penetrate the lymphoid masses. The red pulp contains cells of the erythrocytic line mixed with nongranulated cells and a few granulocytes. Ellipsoids, *i.e.* concentric lamellae of connective tissue around arterioles, are present. Blast cells, probably erythroblasts, and cells with the morphological characteristics of plasma cells are observed in imprint preparations (Fig. 11). Immature red cells with large round nuclei are found in the red pulp of the spleen and in the circulating blood (Fig. 10).

Under electron microscopy granules of spleen granulocytes appear ovoid and contain fibrous inclusions (Fig. 13). Plasma cells are very abundant (Fig. 14).

Discussion

The epigonal organ is the most prominent lymphomyeloid structure in nearly adult specimens of the nurse shark, *Ginglymostoma cirratum*. Curiously enough, Gohar and Mazhar (1964) observed no epigonal organ in an adult female Red Sea nurse shark, *Nebrius concolor*, a species closely related to *Ginglymostoma cirratum*. However, lymphomyeloid organs are dynamic structures that change size and cell composition in relation to endocrine factors (Yoffey and Courtice, 1970). The epigonal organ may have a tendency to involute during maturing of the gonads, although it is not likely to disappear completely.

The main function of the epigonal organ, as indicated by its cell composition, is production and storage of leucocytes, primarily granulocytes. However, it also contains lymphocytes and probably great numbers of plasma cells, although the latter were identified by light microscopy only.

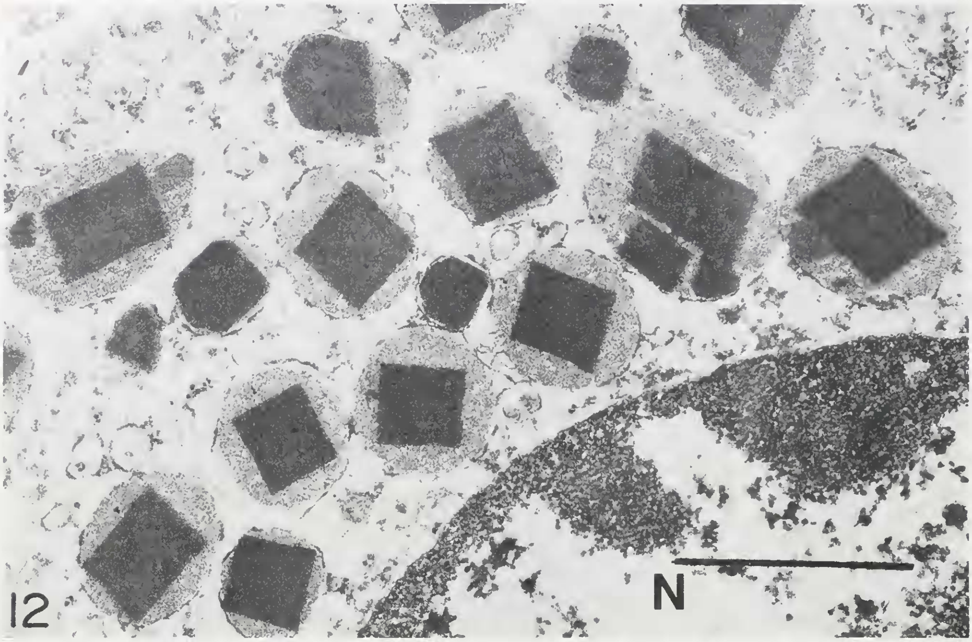
FIGURE 7. MGG-stained imprint of epigonal organ, showing immature granulocytes (promyelocytes). Bar = 10 μm.

FIGURE 8. Peroxidase-containing cells in frozen section of epigonal organ. Bar = 50 μm.

FIGURE 9. MGG-stained blood smear, containing granulocyte with segmented nucleus. Bar = 10 μm.

FIGURE 10. MGG-stained blood smear, showing one mature and one immature erythrocyte. Bar = 10 μm.

FIGURE 11. MGG-stained imprint of the spleen, showing plasma cell. Bar = 10 μm.



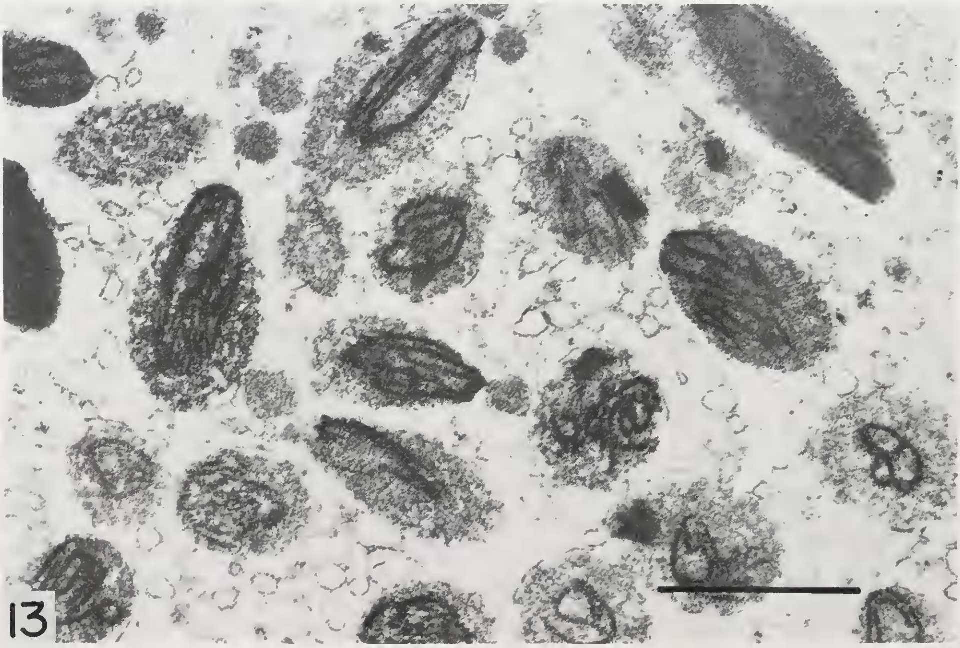


FIGURE 12. Electron micrograph of epigonal organ; part of granulocyte showing granules with rhomboid crystalloid inclusions. Bar = 1 μm. N = nucleus.

FIGURE 13. Electron micrograph of spleen. Granulocyte cytoplasm showing granules with fibrous inclusions. Bar = μm.

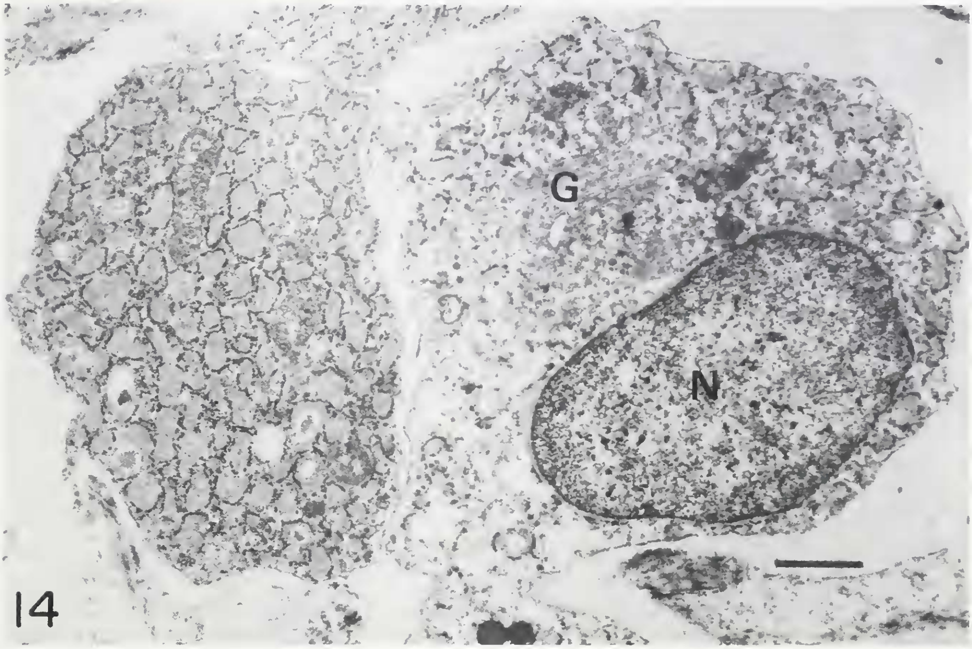


FIGURE 14. Electron micrograph of a plasma cell in the spleen. Bar = 1 μm. G = Golgi apparatus; N = nucleus.

Leucocytes are known to contain hydrolytic lysosomal enzymes. In accordance with this, the epigonal organs of *Ginglymostoma* and other elasmobranchs are rich in glycosidases (Fänge *et al.*, 1980). The finding in the epigonal organ of cells containing peroxidase contrasts with a result by Fey (1966), who found little peroxidase in blood leucocytes of the shark, *Scyliorhinus canicula.* The divergence in results could be a species difference. Leucocytic peroxidases are assumed to take part in bacteria killing systems (*cf.* Selvaraj *et al.*, 1980).

The nurse shark lives close to the bottom in shallow tropical and subtropical inshore waters. In these surroundings, liability to infections and mechanical damage may be greater than in the open sea, necessitating efficient immune and wound-healing mechanisms. Immunological studies have shown that the nurse shark produces two types of immunoglobulins and has a complement system, and that in the adult stage about 50% of its plasma proteins are immunoglobulins (Fidler *et al.*, 1969; Rudikoff *et al.*, 1970; Ross and Jensen, 1973). The blood contains cells similar to mammalian T lymphocytes, and cells cytotoxic towards a variety of target cells (Lopez *et al.*, 1974; McKinney *et al.*, 1977). The nurse shark has a highly differentiated immune system, and efficient wound healing (Reif, 1978). The well developed epigonal organ may supplement the spleen in antibody production, and leucocytes originating in the epigonal organ may function in inflammatory processes during wound healing. In histological structure the epigonal organ rather closely resembles hemopoietic bone marrow, although it produces only white cells. The initial stage of erythropoiesis takes place in the red pulp of the spleen.

Thus Zapata (1980) found ultrastructural evidence of splenic erythropoiesis in rays (*Raja, Torpedo*). However, red cells may mature in the peripheral blood (Kanesada, 1956).

Of the about 600 living species of elasmobranchs (Nelson, 1976), only a few have been examined for appearance and distribution of lymphomyeloid tissues. Studies from our laboratory (Fänge, 1977, and unpublished observations) show an epigonal organ but no Leydig organ in *Ginglymostoma cirratum* (present work), *Rhinoptera bonasus, Heterodontus francisci,* or *Negaprion brevirostris*. Both epigonal and Leydig organs occur in *Raja* species, *Scyliorhinus canicula, Scyliorhinus stellaris,* and *Sequalus acanthias*. Elasmobranchs that possess a Leydig organ but no epigonal organ or only traces of one are *Somniosus microcephalus, Etmopterus spinax,* and *Torpedo* species. In no elasmobranchs are both the epigonal and Leydig organs missing. Hologephalians (chimaeroid cartilaginous fish) lack both epigonal organs and Leydig organs, but have similar lymphomyeloid tissues in cavities of the cartilage skeleton (Stahl, 1967).

ACKNOWLEDGMENTS

Animals were maintained at the Laboratory of Neurobiology, San Juan, Puerto Rico. Thanks are due to the Director, Dr. Jose El Castillo, his staff, and Dr. Sven Ebbeson, for good working facilities. I thank Dr. P. Leuken for advice on histochemistry and Ms. Lolyn Lopez for expert technical assistance. This work was supported by the Swedish National Science Research Council.

LITERATURE CITED

CLARK, E. 1963. The maintenance of sharks in captivity, with a report in their instrumental conditioning. Pp. 115–149 in P. W. Gilberts, Ed., *Sharks and Survival*. D. C. Heath and Co., Boston.

DE OLMOS, J., AND L. HEIMER. 1977. Mapping of collateral projections with the horseradish peroxidase method. *Neurosci. Lett.* **6:** 107–114.

FÄNGE, R. 1977. Size relations of lymphomyeloid organs in some cartilaginous fish. *Acta Zool. (Stockholm)* **58:** 125–128.

FÄNGE, R., G. LUNDBLAD, K. SLETTENGREN, AND J. LIND. 1980. Glycosidases in lymphomyeloid (hematopoietic) tissues of elasmobranch fishes. *Comp. Biochem. Physiol.* **67B:** 527–532.

FEY, F. 1966. Vergleichende Hämozytologie niederer Vertebraten. III. Granulozyten. *Folia Haematol.* **86:** 1–20.

FIDLER, J. E., L. W. CLEM, AND P. A. SMALL, JR. 1969. Immunoglobulin synthesis in neonatal nurse shark (*Ginglymostoma cirratum*). *Comp. Biochem. Physiol.* **31:** 365–371.

GOHAR, H. A. F., AND F. M. MAZHAR. 1964. Internal anatomy of selachians from the northwestern Red Sea. *Publications of the Marine Biological Station Al-Ghardaqa (Egypt)* **13:** 145–240.

KANESADA, A. 1956. A phylogenetic survey of hemocytopoietic tissues in submammalian vertebrates. *Bull. Yamaguchi Med. Sch.* **4:** 1–21.

LOPEZ, D. M., M. M. SIGEL, AND J. C. CLEM. 1974. Phylogenetic studies on T cells. 1. Lymphocytes of the shark with differential response to phytohemagglutinin and concanavalin A. *Cell. Immunol.* **10:** 287–293.

MACKINNEY, E. C., C. L. PETTEY, D. M. LOPEZ, AND M. M. SIGEL. 1977. Cytotoxicity of nurse shark (*Ginglymostoma cirratum*) leucocytes. Pp. 217–224 in A. E. Ellis, J. B. Solomon, and J. D. Hoston, Eds., *Developmental Immunology*. Elsevier, New York.

MATTHEWS, L. H. 1950. Reproduction in the basking shark, *Cetorhinus maximus* (Gunner). *Phil. Trans. R. Soc. Lond. B. Biol. Sci.* **234:** 247–299.

NELSON, J. S. 1976. *Fishes of the World*. John Wiley and Sons, New York. 1416 pp.

REIF, W.-E. 1978. Wound healing in sharks. Form and arrangement of repair scales. *Zoomorphologie* **90:** 101–111.

ROSS, G. D., AND J. A. JENSEN. 1973. The first component (C in) of the complement system of the nurse shark (*Ginglymostoma cirratum*). I. Hemolytic characteristics of partially purified C in. *J. Immunol.* **110:** 175–182.

RUDIKOFF, S., E. W. VOSS, AND M. M. SIGEL. 1970. Biological and chemical properties of natural antibodies in the nurse shark, *Ginglymostoma cirratum. J. Immunol.* **105:** 1344–1352.

SELVARAJ, R. J., J. M. ZGLICZYNSKI, B. B. PAUL, AND A. J. SBARRA. 1980. Chlorination of reduced nicotinamide adenine nucleotide by myeloperoxidase. A novel bactericidal mechanism. *J. Reticuloendothelial Soc.* **27:** 31–38.

STAHL, B. J. 1967. Morphology and relationships of the Holocephali with special reference to the venous sytsem. *Bull. Mus. Comp. Zool.* **135:** 141–213.

YOFFEY, J. M., AND F. C. COURTICE. 1970. *Lymphatics, Lymph and the Lymphomyeloid Complex.* Academic Press, New York. 942 pp.

ZAPATA, A. 1980. Splenic erythropoiesis and thrombopoiesis in elasmobranchs. An ultrastructural study. *Acta Zool. (Stockholm)* **61:** 59–64.